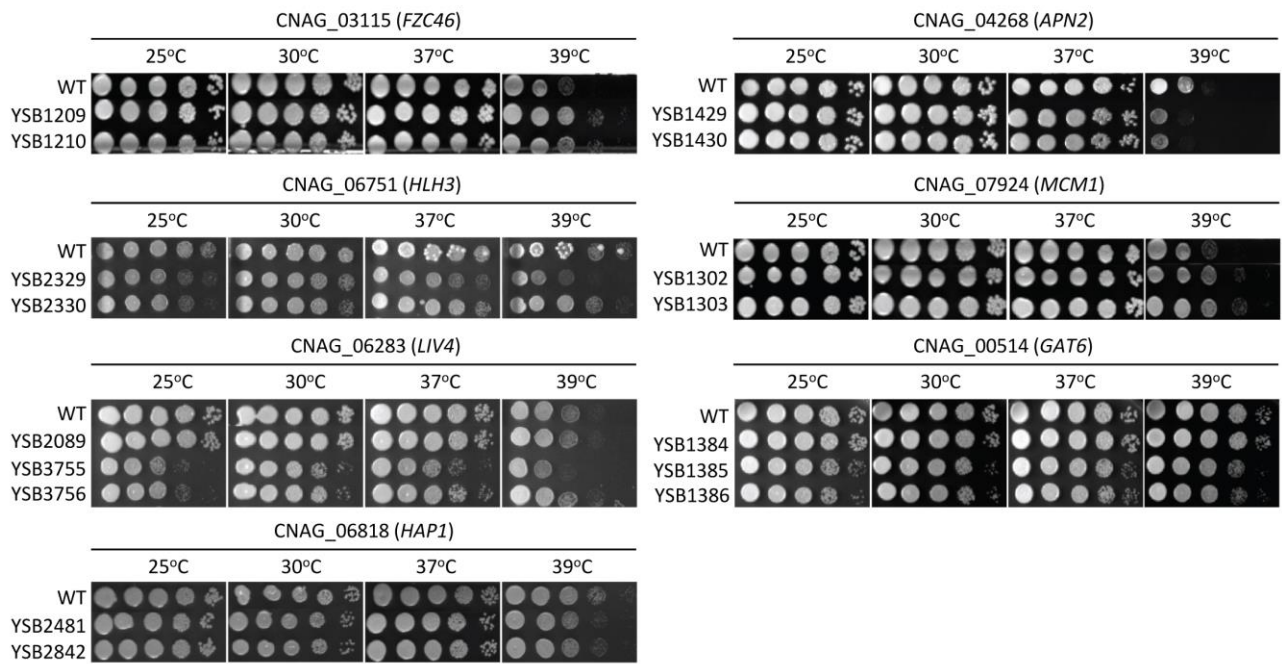
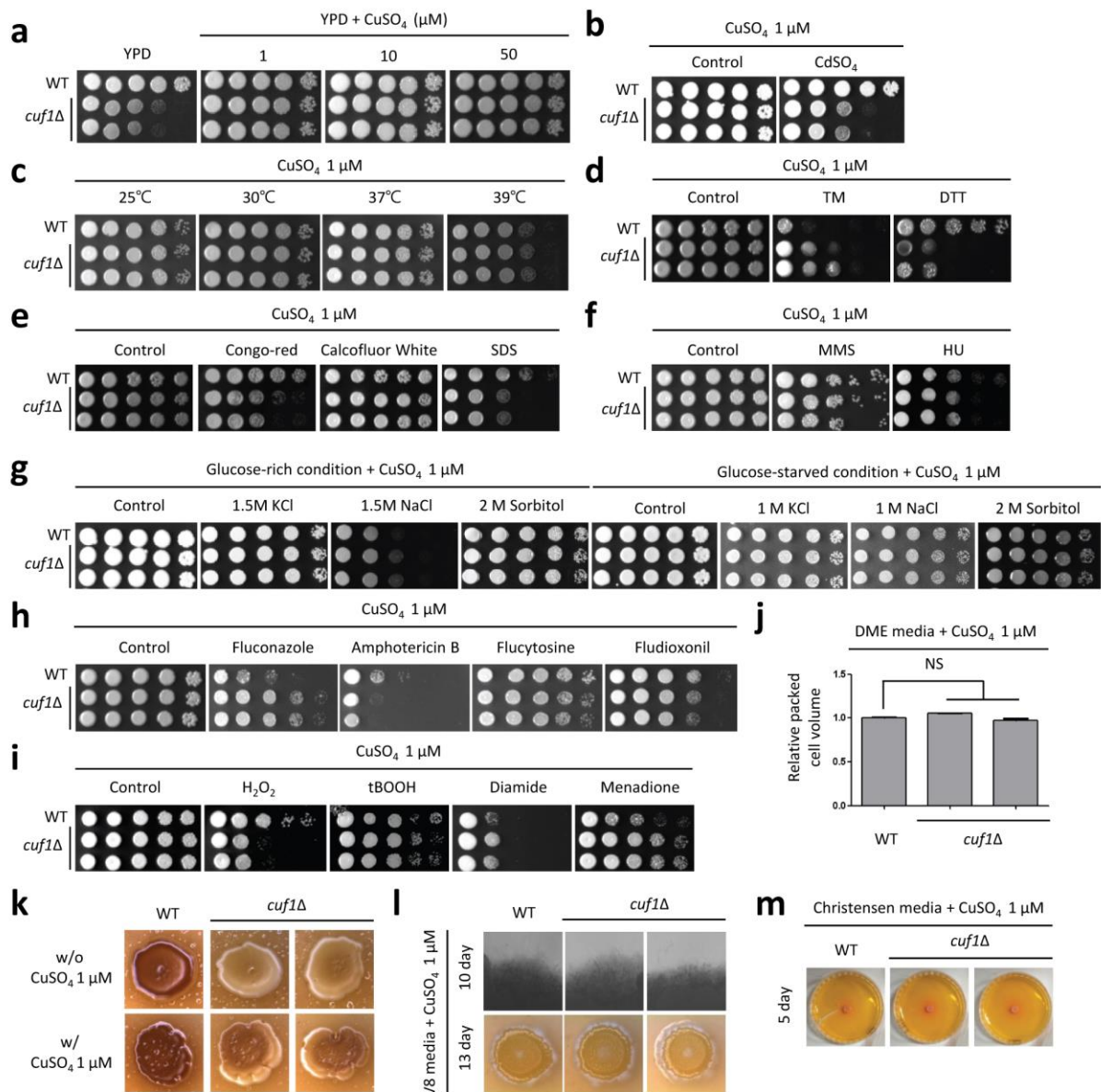


*Continued*



**Supplementary Figure 1: Transcription factors required for temperature-dependent growth of *C. neoformans*.** *C. neoformans* strains were grown overnight in liquid YPD medium at 30°C, serially diluted (1 to 10<sup>4</sup> dilutions), and spotted (3 µl of each dilution) onto YPD agar medium. Cells were incubated at 25, 30, 37, and 39°C for 3 days and then photographed.



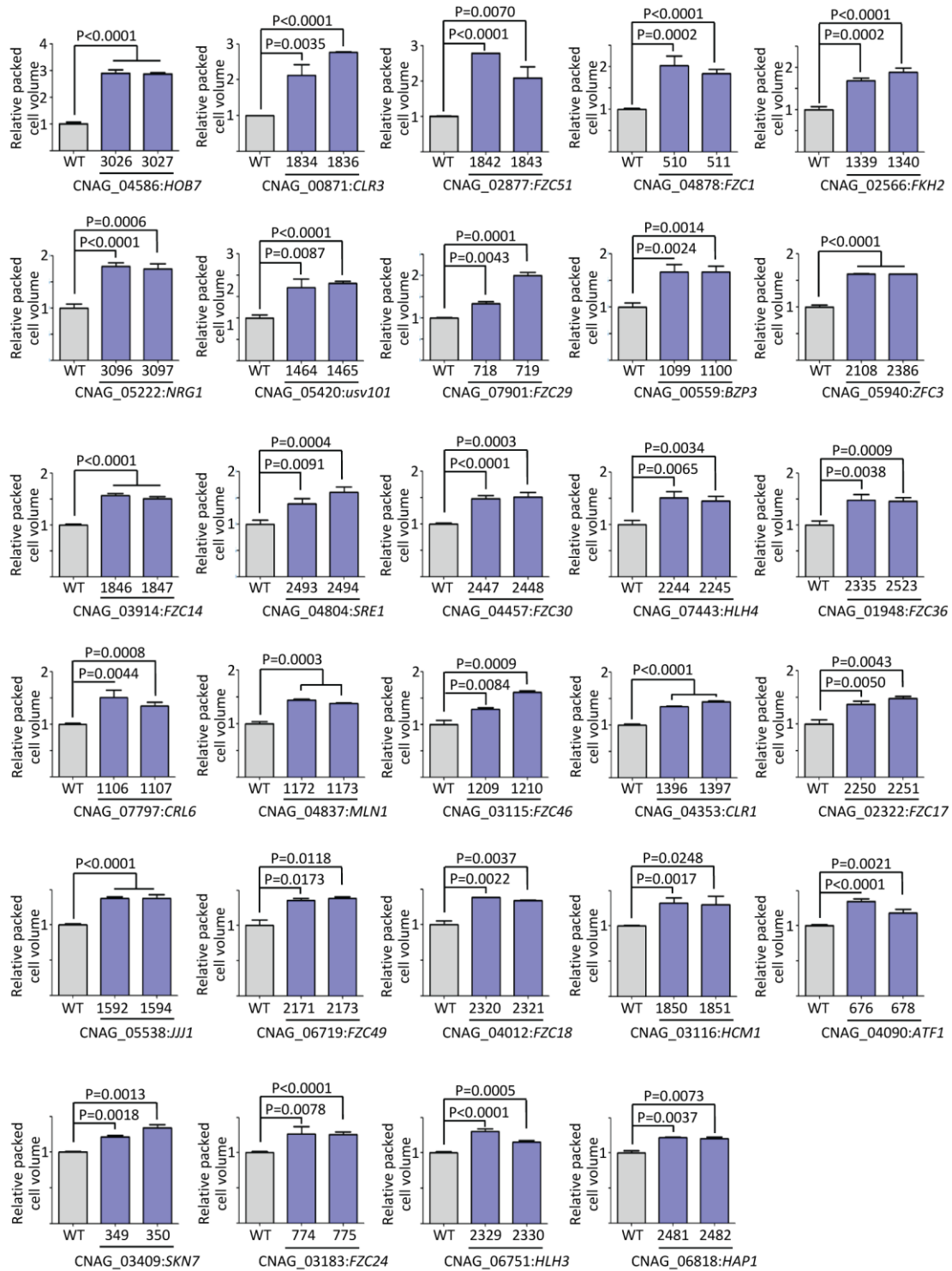
**Supplementary Figure 2: The role of Cuf1 and copper uptake in the growth, differentiation, stress responses, antifungal drug susceptibility, and virulence-factor production of *C. neoformans*.**

(a) WT (H99) and *cuf1Δ* (YSB2665 and YSB2666) mutants were spotted onto YPD media containing the indicated concentrations of CuSO<sub>4</sub>. Cells were grown at 30°C and photographed after growth for 2 days. (b, d–i) WT and *cuf1Δ* mutants were grown in liquid YPD media for 16 h at 30°C, 10-fold serially diluted (1 to 10<sup>4</sup> dilutions), and spotted (3 μl of each dilution) onto YPD (glucose-rich condition) or YP (glucose-starved condition) agar medium containing 1 μM CuSO<sub>4</sub> and the following stress inducers: CdSO<sub>4</sub> 30 μM (b), TM 0.3 μg ml<sup>-1</sup> (c), DTT 15 mM (d), Congo red 0.8% (e), calcofluor white 3 mg ml<sup>-1</sup> (e), SDS 0.03% (e), MMS 0.03% (f), HU 100 mM (f), NaCl/KCl 1.5 M or sorbitol 2 M (g), fluconazole 14 μg ml<sup>-1</sup> (h), amphotericin B 0.8 μg ml<sup>-1</sup> (h), flucytosine 300 μg ml<sup>-1</sup> (h), fludioxonil 1 μg ml<sup>-1</sup> (h), H<sub>2</sub>O<sub>2</sub> 3.5 mM (i), tBOOH 0.8 mM (i), diamide 2.5 mM (i), and menadione 0.02 mM (i). Cells were further incubated at 30°C and photographed daily for 2–4 days. (c) WT and *cuf1Δ* mutants were spotted onto YPD media containing 1 μM CuSO<sub>4</sub> and incubated at the indicated temperatures for 2 days. (j–m) To perform the capsule, melanin, mating, and urease assays, cells were spotted or incubated in agar-based Dulbecco's Modified Eagle's (DME) medium, Niger seed medium, V8 medium, and Christensen's agar medium, respectively, containing 1 μM CuSO<sub>4</sub>.

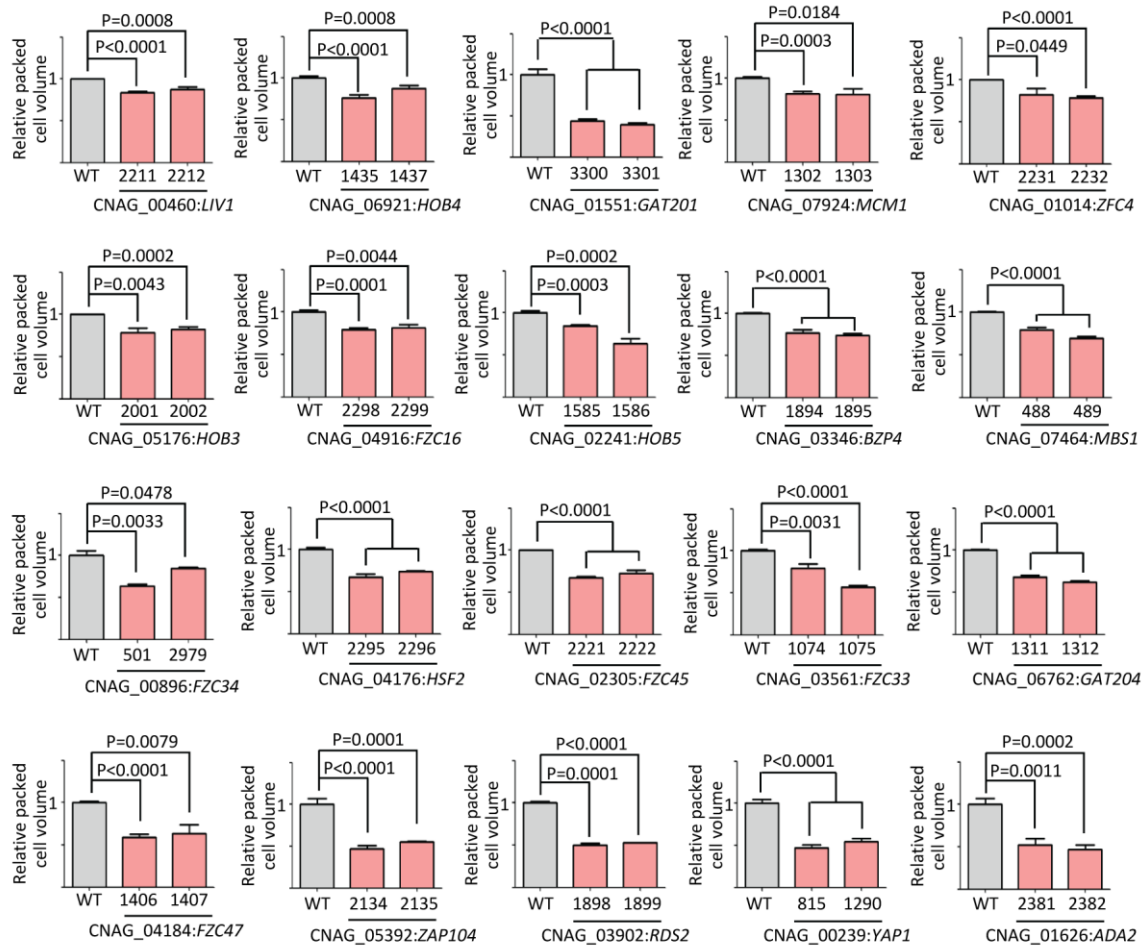




**Supplementary Figure 3: TF mutants involved in the sexual differentiation of *C. neoformans*.** In the mating assay, the wild-type strain H99 (WT) and each TF mutant were cocultured with the opposite mating type KN99a strain on V8 media and incubated at room temperature in the dark for 11 days and then photographed using a microscope (Motic Microscope) equipped with a digital camera (Pro-Microscan No. 5888). (a) and (b) panels include groups of TF mutants showing enhanced and decreased mating efficiency, respectively.

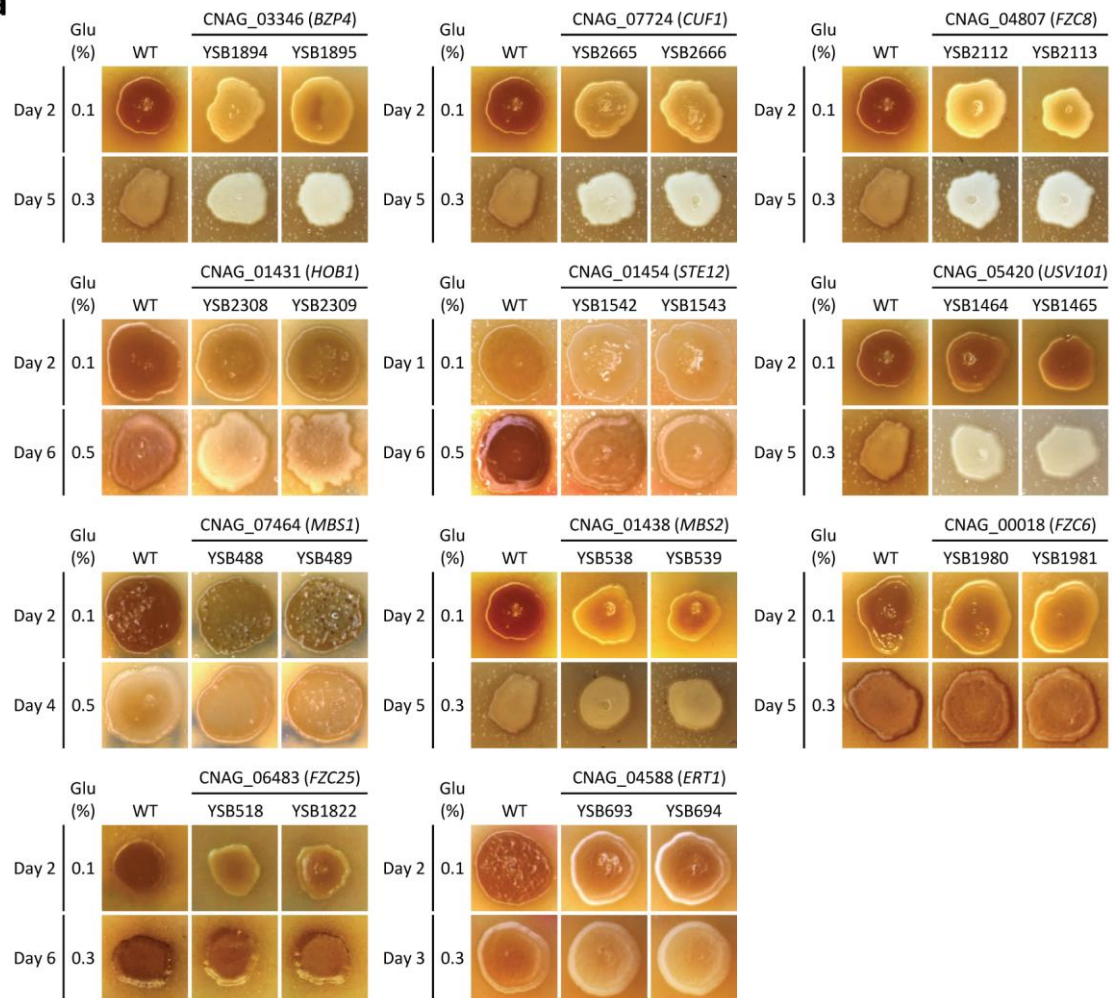
**a****Continued**

b



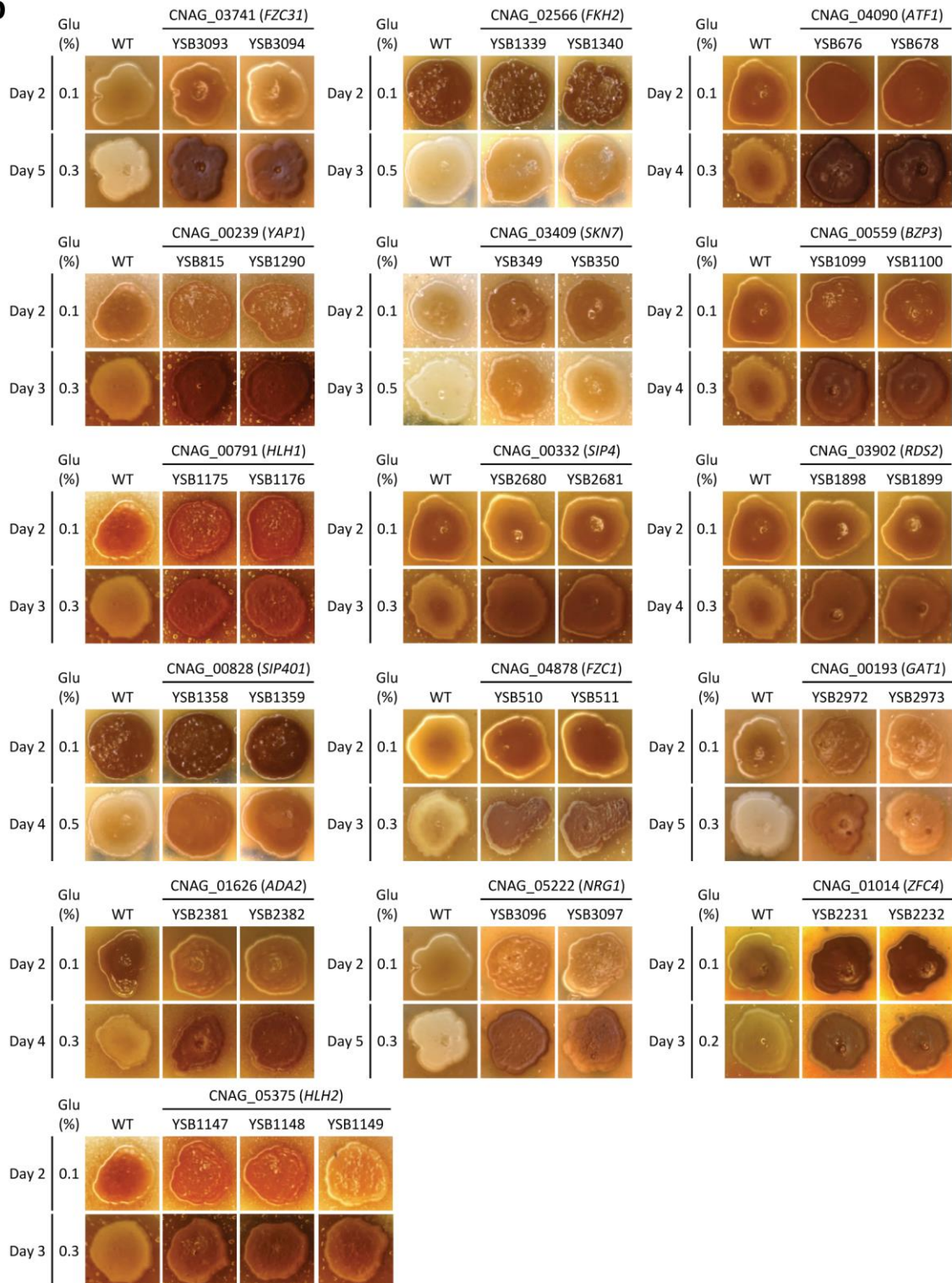
#### Supplementary Figure 4: TF mutants involved in capsule production in *C. neoformans*. To

quantify capsule production, each strain was grown in YPD medium for 16 h at 30°C, spotted on agar-based DME, and incubated at 37°C for 2 days. The cells were then scraped, fixed, and synchronized to equal concentration ( $5 \times 10^8$  cells  $\text{ml}^{-1}$ ). An equal number of cells ( $2.5 \times 10^7$  cells  $\text{ml}^{-1}$ ) of each strain was injected into a hematocrit capillary tube and allowed to precipitate by gravity after 10 days. The packed cell volume ratio was measured by calculating the ratio of the length of the packed cell volume phase to the length of the total volume phase (cells + medium) in cryptocrit. The relative packed cell volume of each mutant was measured by calculating the ratio of the mutant packed cell volume ratio to the WT packed cell volume ratio. Three independent technical replicate tests were performed using two independent strains. For statistical tests, one-way ANOVA with Bonferroni's posttest was performed for selected pairs of WT and mutant strains by using Prism 5 (Graph pad software). Two independent TF mutants that are statistically different ( $P < 0.05$ ) are shown in this figure. (a) and (b) panels include groups of TF mutants showing enhanced and decreased capsule production, respectively.

**a*****Continued***

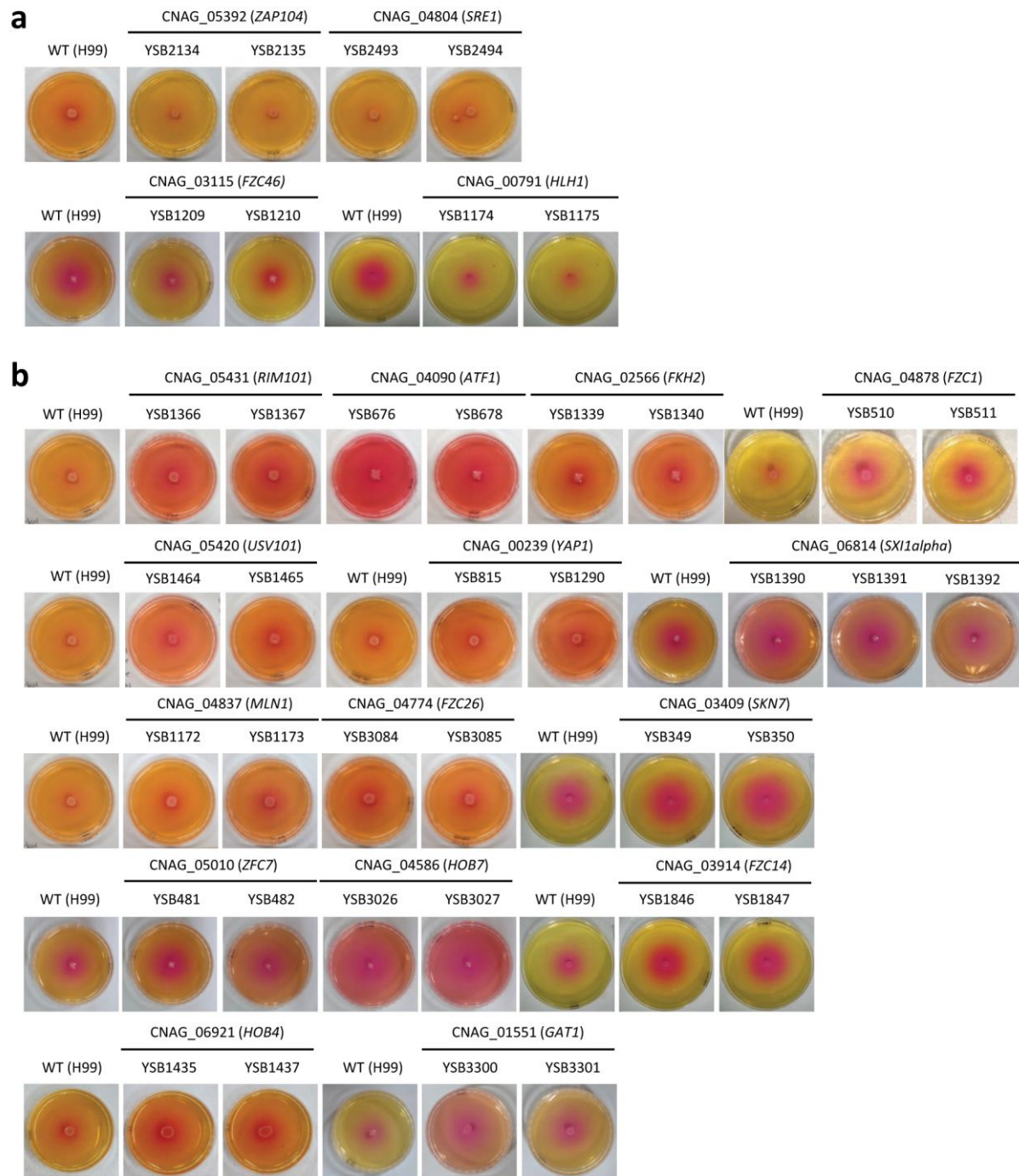


**b**



**Supplementary Figure 5: TF mutants involved in melanin production in *C. neoformans*.** *C. neoformans* strains were cultured overnight in liquid YPD medium at 30°C, after which 5 µl of cultures were spotted on Niger seed agar media containing the indicated concentration of glucose and further incubated at 37°C. (a) and (b) panels include groups of TF mutants showing reduced and enhanced melanin production, respectively

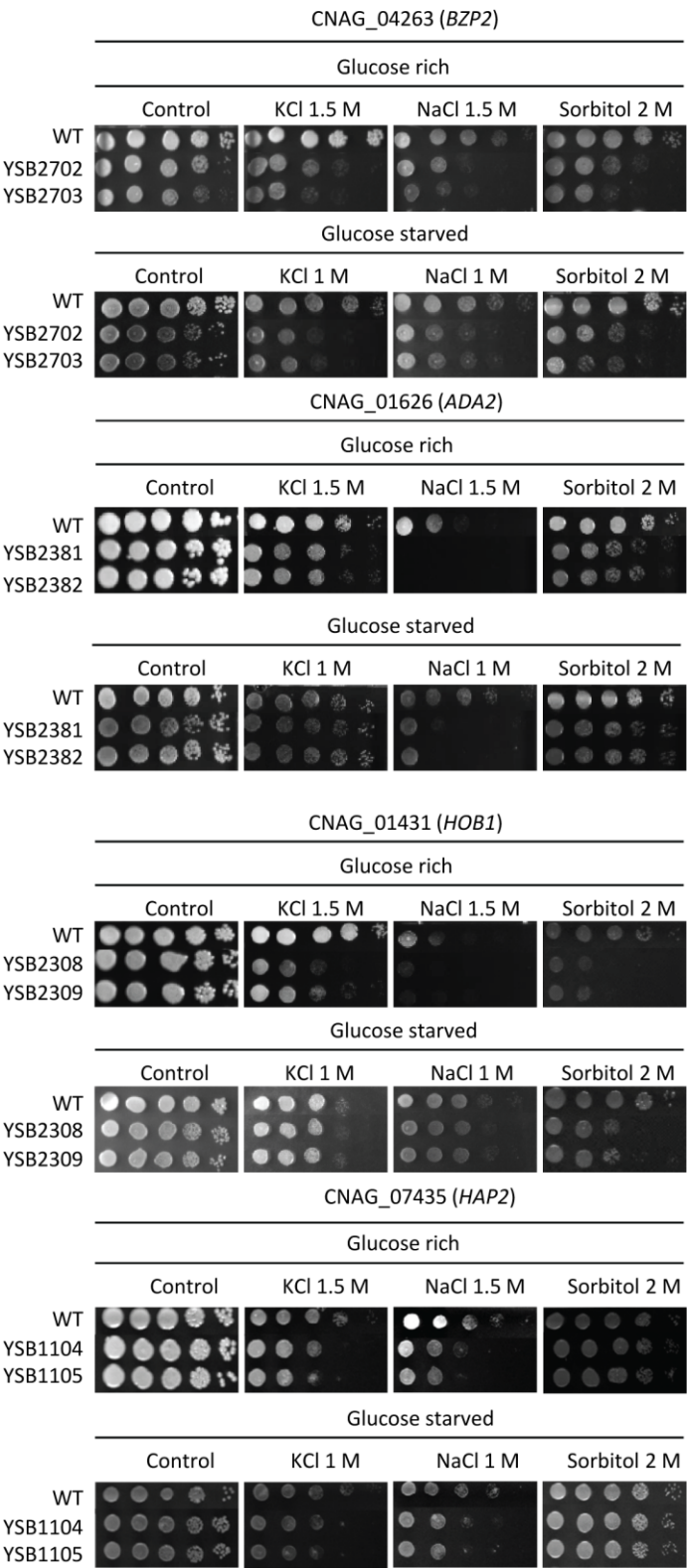




**Supplementary Figure 6: TFs required for urease production in *C. neoformans*.** *C. neoformans* strains were grown overnight in liquid YPD medium at 30°C. Cells were washed with distilled water and adjusted to  $1 \times 10^7$  cells  $\text{ml}^{-1}$  and then 5  $\mu\text{l}$  of cells ( $5 \times 10^4$  cells) were spotted onto Christensen's agar media and further incubated for 7–10 days at 30°C. (a) and (b) panels include groups of TF mutants showing reduced and enhanced urease production, respectively.

**a** TFs involved in osmotic stress responses

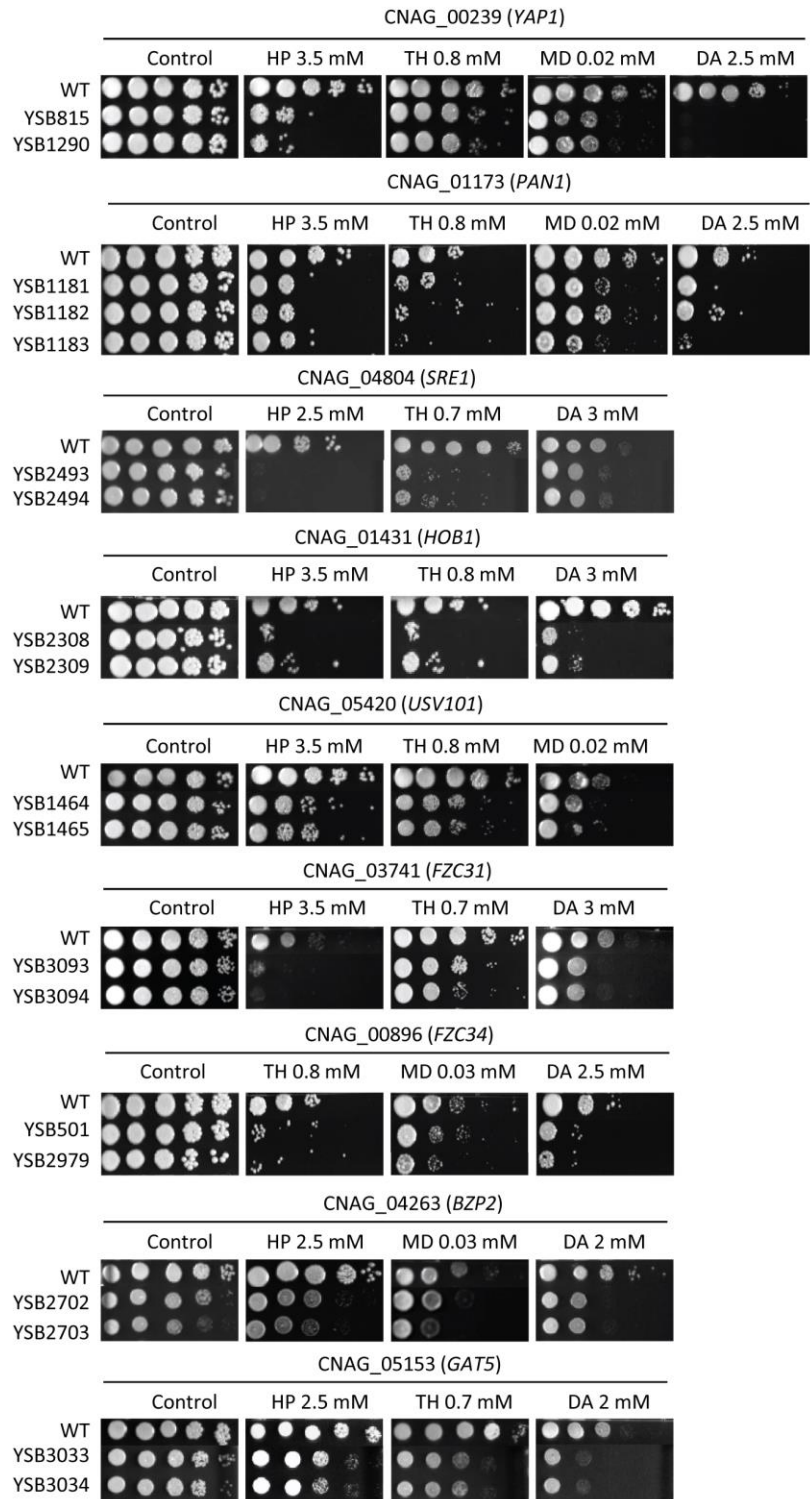
	H99 ID	Designated name	NR	KR	SR	NS	KS	SS
1	04263	<i>BZP2</i>						
2	01626	<i>ADA2</i>						
3	01431	<i>HOB1</i>						
4	07435	<i>HAP2</i>						
5	05431	<i>RIM101</i>						
6	01948	<i>FZC36</i>						
7	00018	<i>FZC6</i>						
8	07464	<i>MBS1</i>						
9	03409	<i>SKN7</i>						
10	05153	<i>GAT5</i>						
11	01173	<i>PAN1</i>						
12	05170	<i>PIP2</i>						
13	00039	<i>ZFC6</i>						
14	05380	<i>FZC44</i>						
15	03741	<i>FZC31</i>						
16	05093	<i>HOB6</i>						
17	03116	<i>HCM1</i>						
18	04841	<i>FZC43</i>						
19	02066	<i>FZC13</i>						
20	01708	<i>GAT7</i>						
21	07724	<i>CUF1</i>						
22	06751	<i>HLH3</i>						
23	05222	<i>NRG1</i>						
24	04345	<i>ARO8001</i>						
25	00068	<i>MET32</i>						
26	04630	<i>YAP2</i>						
27	03115	<i>FZC46</i>						
28	00896	<i>FZC34</i>						
29	03527	<i>HEL2</i>						
30	05112	<i>FZC42</i>						
31	01883	<i>GAT8</i>						
32	03346	<i>BZP4</i>						
33	06339	<i>FZC35</i>						
34	02364	<i>FZC19</i>						
35	02877	<i>FZC51</i>						
36	03768	<i>FZC32</i>						



*Continued*

## b TFs involved in oxidative stress responses

H99 ID	Designated name	HP	TH	MD	DA
1	00239	YAP1			
2	01173	PAN1			
3	04804	SRE1			
4	01431	HOB1			
5	05420	USV101			
6	03741	FZC31			
7	00896	FZC34			
8	07724	CUF1			
9	04263	BZP2			
10	05153	GAT5			
11	00068	MET32			
12	04594	FZC27			
13	05019	FZC21			
14	01973	FZC2			
15	07922	FZC4			
16	00018	FZC6			
17	05222	NRG1			
18	05093	HOB6			
19	05431	RIM101			
20	00791	HLH1			
21	03710	ECM22			
22	04630	YAP2			
23	03115	FZC46			
24	07435	HAP2			
25	06921	HOB4			
26	07940	BZP5			
27	00514	GAT6			
28	05538	JUJ1			
29	03409	SKN7			
30	06339	FZC35			
31	07506	FAP1			
32	02435	BWC2			
33	02364	FZC19			
34	05380	FZC44			
35	00460	LIV1			
36	02305	FZC45			
37	06719	FZC49			
38	01626	ADA2			
39	04895	FZC3			
40	02066	FZC13			
41	00193	GAT1			
42	03336	FZC50			
43	00830	FZC38			
44	06223	MIZ1			
45	03059	FZC9			
46	04807	FZC8			
47	06751	HLH3			
48	02566	FKH2			
49	07464	MBS1			
50	03561	FZC33			
51	06762	GAT204			
52	01438	MBS2			
53	04837	MLN1			
54	05642	FZC37			
55	04878	FZC1			
56	06425	PPR1			
57	04090	ATF1			
58	07924	MCM1			
59	02555	SIP402			
60	06188	FZC15			
61	05170	PIP2			
62	02241	HOB5			
63	05186	GRF1			
64	01454	STE12			
65	03527	HEL2			
66	01883	GAT8			
67	04353	CLR1			
68	05375	HLH2			
69	03998	RLM1			
70	00156	SPI1(CRZ1)			
71	07011	FZC22			
72	03116	HCM1			
73	02877	FZC51			
74	00559	BZP3			
75	00871	CLR3			
76	03849	ASG1			
77	01014	ZFC4			
78	01858	HOB2			
79	04093	YRM103			
80	04176	HSF2			
81	04352	ZAP103			
82	04583	DDT1			
83	06156	FZC7			
84	00332	SIP4			
85	03366	ZNF2			
86	04774	FZC26			
87	03086	FZC20			
88	03229	YOX101			
89	01841	GLN3			
90	02700	ZFC8			
91	04457	FZC30			
92	07411	RUM1			
93	01551	GAT201			

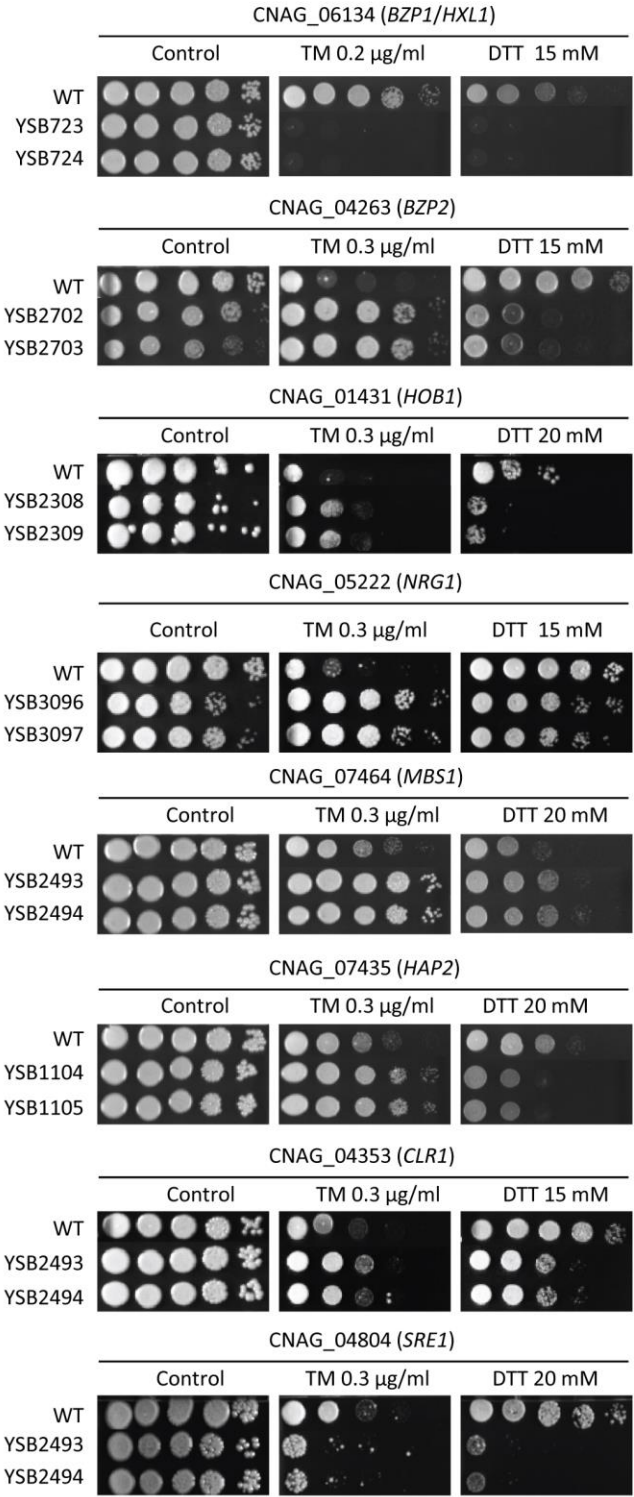


Continued



# C TFs involved in ER stress responses

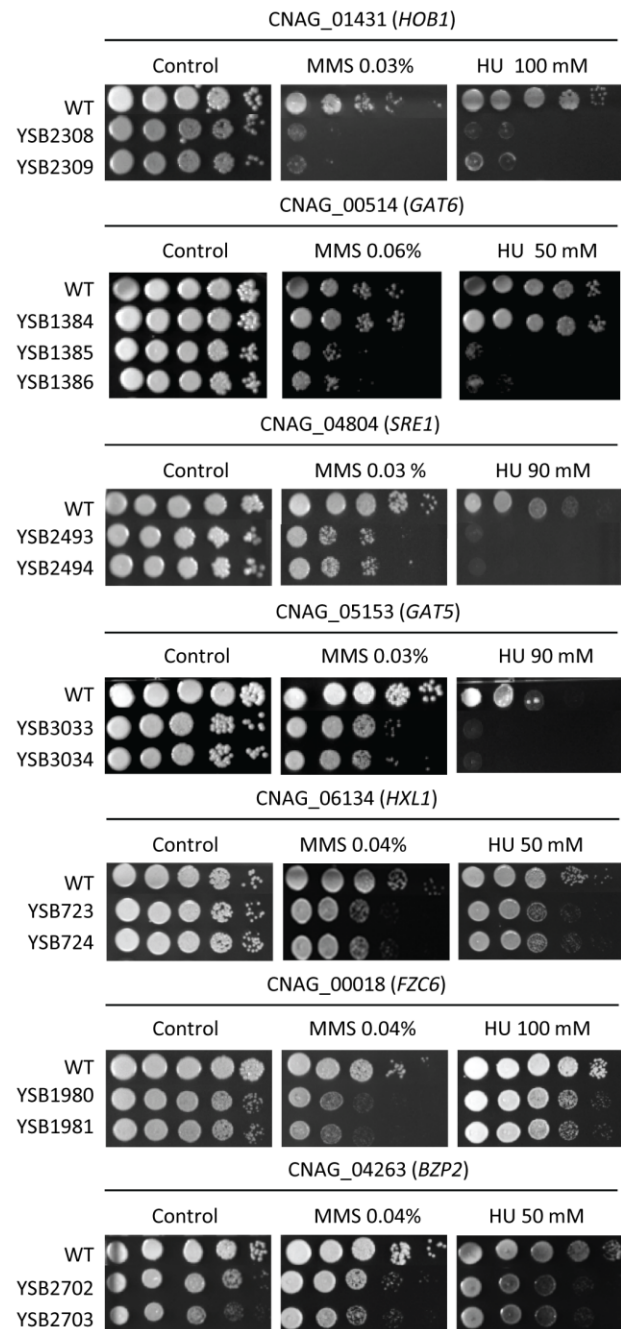
	H99 ID	Designated name	TM	DT
1	06134	<i>BZP1(HXL1)</i>		
2	04263	<i>BZP2</i>		
3	07724	<i>CUF1</i>		
4	01431	<i>HOB1</i>		
5	05222	<i>NRG1</i>		
6	07464	<i>MBS1</i>		
7	07435	<i>HAP2</i>		
8	04353	<i>CLR1</i>		
9	04804	<i>SRE1</i>		
10	05153	<i>GAT5</i>		
11	00791	<i>HLH1</i>		
12	00068	<i>MET32</i>		
13	01173	<i>PAN1</i>		
14	05170	<i>PIP2</i>		
15	03998	<i>RLM1</i>		
16	00559	<i>BZP3</i>		
17	05375	<i>HLH2</i>		
18	04630	<i>YAP2</i>		
19	00018	<i>FZC6</i>		
20	06283	<i>LIV4</i>		
21	02566	<i>FKH2</i>		
22	01069	<i>FZC11</i>		
23	03401	<i>GAT203</i>		
24	00828	<i>SIP401</i>		
25	05785	<i>STB4</i>		
26	07593	<i>YAP4</i>		
27	04837	<i>MLN1</i>		
28	05431	<i>RIM101</i>		
29	06425	<i>PPR1</i>		
30	01454	<i>STE12</i>		
31	03527	<i>HEL2</i>		
32	05255	<i>FZC2</i>		
33	00239	<i>YAP1</i>		
34	00156	<i>SP1(CRZ1)</i>		
35	04268	<i>APN2</i>		
36	05420	<i>USV101</i>		
37	00514	<i>GAT6</i>		
38	05538	<i>JJJ1</i>		
39	03409	<i>SKN7</i>		
40	02435	<i>BWC2</i>		
41	03116	<i>HCM1</i>		
42	06483	<i>FZC25</i>		
43	05019	<i>FZC21</i>		
44	05380	<i>FZC44</i>		
45	05176	<i>HOB3</i>		
46	01014	<i>ZFC4</i>		
47	01626	<i>ADA2</i>		
48	05940	<i>ZFC3</i>		
49	01948	<i>FZC36</i>		
50	04583	<i>DDT1</i>		
51	01973	<i>ZFC2</i>		
52	01708	<i>GAT7</i>		
53	03902	<i>RDS2</i>		
54	00193	<i>GAT1</i>		
55	03086	<i>FZC20</i>		
56	02700	<i>ZFC8</i>		
57	04586	<i>HOB7</i>		
58	03741	<i>FZC31</i>		
59	01551	<i>GAT201</i>		
60	04908	<i>CLR4</i>		



Continued

# d TFs involved in genotoxic stress responses

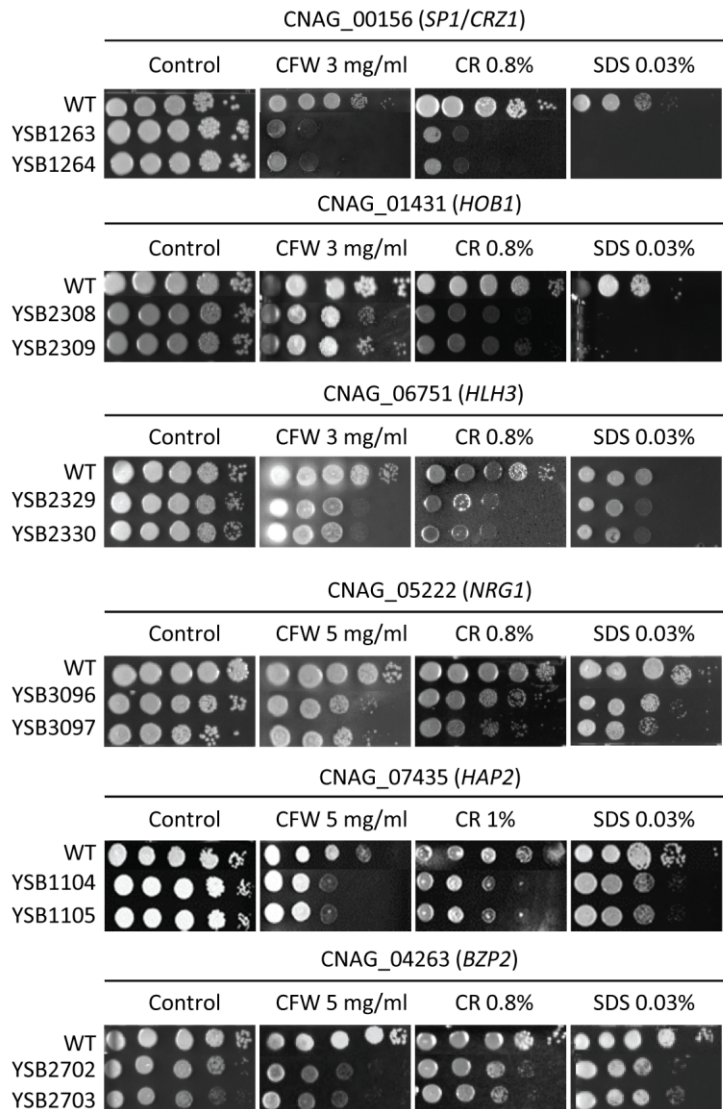
	H99 ID	Designated name	MS	HU
1	01431	<i>HOB1</i>		
2	00514	<i>GAT6</i>		
3	04804	<i>SRE1</i>		
4	05153	<i>GAT5</i>		
5	06134	<i>BZP1(HXL1)</i>		
6	00018	<i>FZC6</i>		
7	04263	<i>BZP2</i>		
8	05538	<i>JJJ1</i>		
9	06223	<i>MIZ1</i>		
10	05222	<i>NRG1</i>		
11	07464	<i>MBS1</i>		
12	04878	<i>FZC1</i>		
13	05170	<i>PIP2</i>		
14	05375	<i>HLH2</i>		
15	04268	<i>APN2</i>		
16	03409	<i>SKN7</i>		
17	03116	<i>HCM1</i>		
18	03212	<i>HCM101</i>		
19	01626	<i>ADA2</i>		
20	07922	<i>FZC4</i>		
21	03086	<i>FZC20</i>		
22	03229	<i>YOX101</i>		



*Continued*

## e TFs involved in cell wall/membrane stress responses

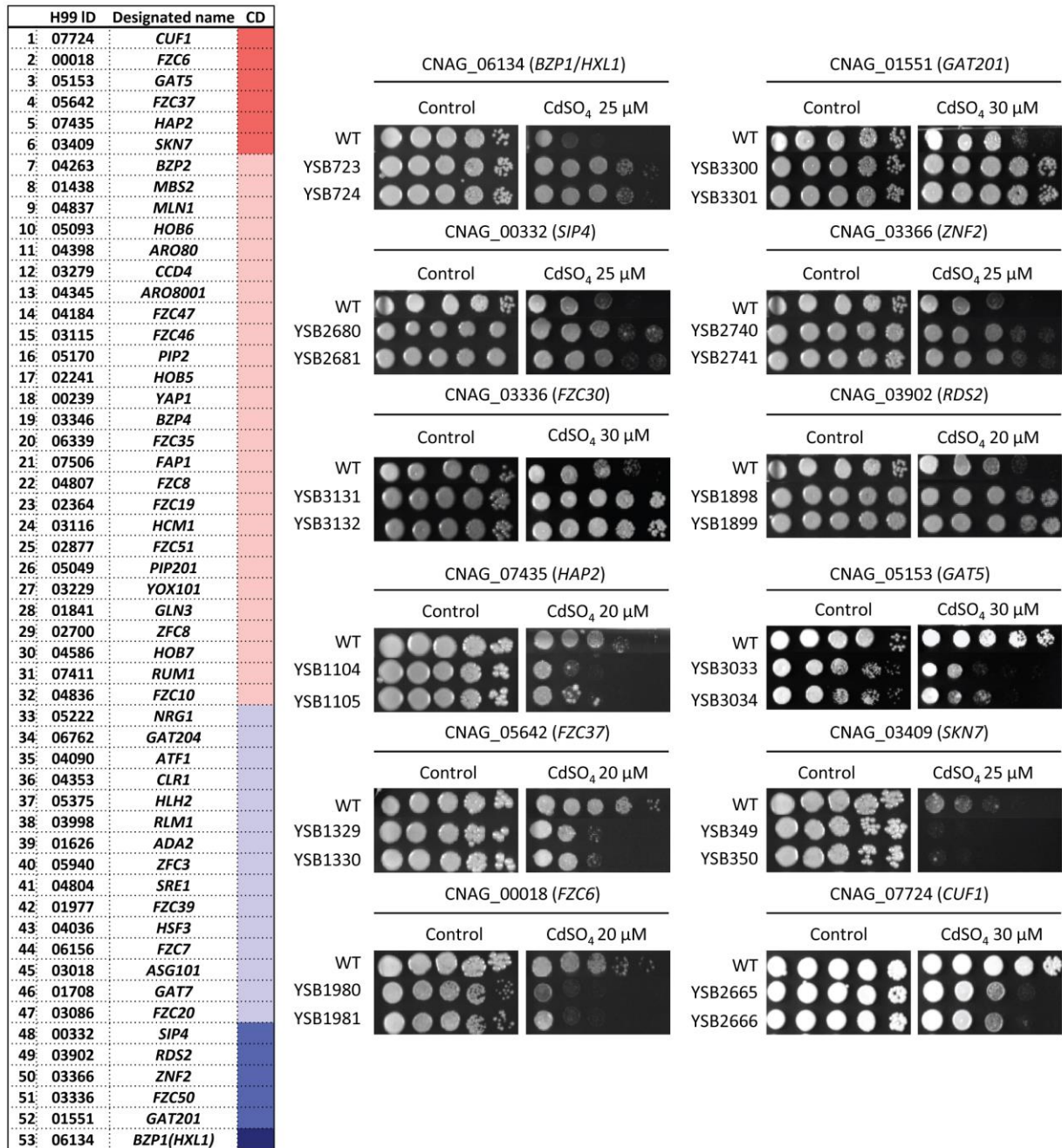
	H99 ID	Designated name	CW	CR	SD
1	00156	<i>SP1(CRZ1)</i>			
2	01431	<i>HOB1</i>			
3	06751	<i>HLH3</i>			
4	05222	<i>NRG1</i>			
5	07435	<i>HAP2</i>			
6	04263	<i>BZP2</i>			
7	06134	<i>BZP1(HXL1)</i>			
8	07724	<i>CUF1</i>			
9	05431	<i>RIM101</i>			
10	04630	<i>YAP2</i>			
11	00018	<i>FZC6</i>			
12	05153	<i>GAT5</i>			
13	04588	<i>ERT1</i>			
14	04878	<i>FZC1</i>			
15	03710	<i>ECM22</i>			
16	01173	<i>PAN1</i>			
17	06188	<i>FZC15</i>			
18	05170	<i>PIP2</i>			
19	02241	<i>HOB5</i>			
20	05186	<i>GRF1</i>			
21	00039	<i>ZFC6</i>			
22	07940	<i>BZP5</i>			
23	06814	<i>SXI1alpha</i>			
24	04353	<i>CLR1</i>			
25	03998	<i>RLM1</i>			
26	00239	<i>YAP1</i>			
27	05420	<i>USV101</i>			
28	00514	<i>GAT6</i>			
29	05538	<i>JJJ1</i>			
30	03409	<i>SKN7</i>			
31	07011	<i>FZC22</i>			
32	07506	<i>FAP1</i>			
33	04807	<i>FZC8</i>			
34	02435	<i>BWC2</i>			
35	02877	<i>FZC51</i>			
36	00559	<i>BZP3</i>			
37	07797	<i>CRL6</i>			
38	05019	<i>FZC21</i>			
39	05176	<i>HOB3</i>			
40	03849	<i>ASG1</i>			
41	01014	<i>ZFC4</i>			
42	04804	<i>SRE1</i>			
43	02603	<i>ZFC1</i>			
44	04036	<i>HSF3</i>			
45	06156	<i>FZC7</i>			
46	03018	<i>ASG101</i>			
47	01708	<i>GAT7</i>			
48	00332	<i>SIP4</i>			
49	03902	<i>RDS2</i>			
50	04774	<i>FZC26</i>			
51	03059	<i>FZC9</i>			
52	00193	<i>GAT1</i>			
53	03336	<i>FZC50</i>			
54	02723	<i>FZC23</i>			
55	03741	<i>FZC31</i>			
56	04457	<i>FZC30</i>			
57	07411	<i>RUM1</i>			
58	01551	<i>GAT201</i>			
59	04908	<i>CLR4</i>			



Continued

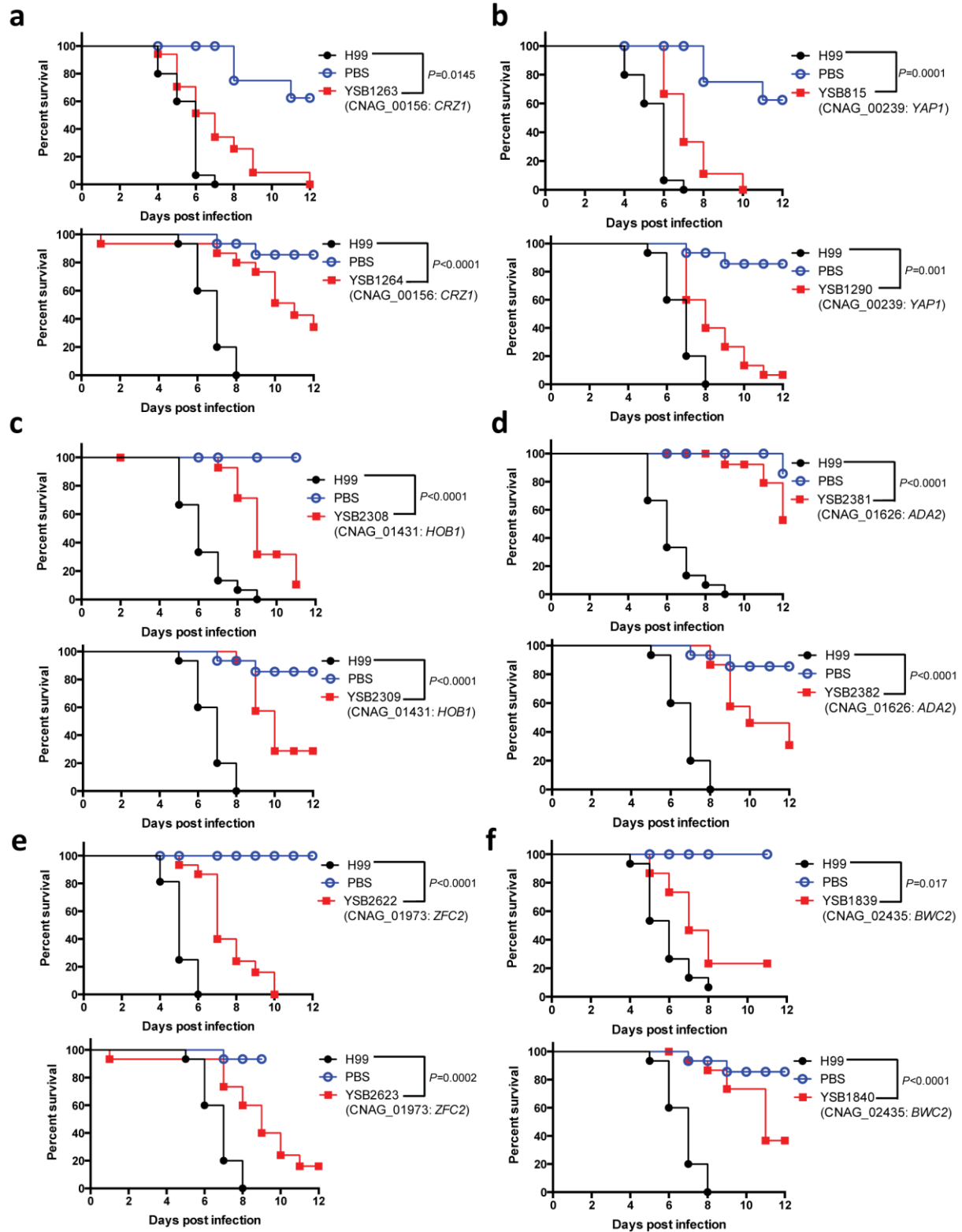


## f TFs involved in heavy metal stress responses

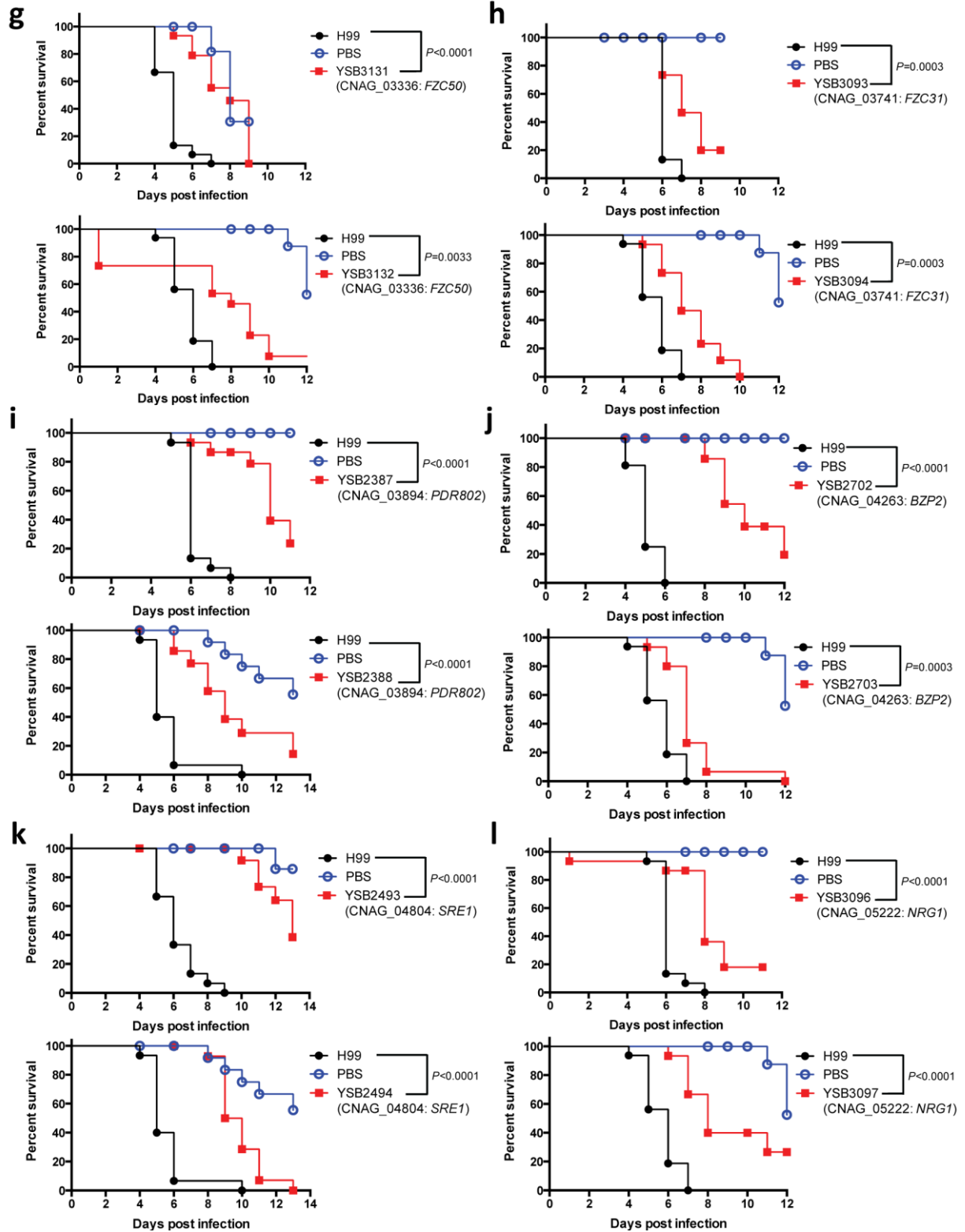


### Supplementary Figure 7. Selected list of TFs involved in stress response and adaption of *C. neoformans*.

*C. neoformans* strains were cultured for 16 h in liquid medium at 30°C. Cells were washed with distilled water and 10-fold serially diluted (1 to 10<sup>4</sup> dilutions) and then spotted (3 µl of each dilution) onto YPD containing the indicated concentrations of stress inducing reagents. a: Osmotic stress; b: Oxidative stress (HP (H<sub>2</sub>O<sub>2</sub>), TH (*tert*-butyl hydroperoxide), MD (menadione), DA (diamide)); c: ER stress (TM (tunicamycin), DTT (dithiothreitol)); d: Genotoxic stress (MMS (methyl methanesulfonate), HU (hydroxyurea)); e: Cell wall/membrane stress (CFW (calcofluor white), CR (Congo red), SDS (sodium dodecyl sulfate)); and f: Heavy metal stress. In the left side of each panel, the phenome heat map for each stress response was indicated.

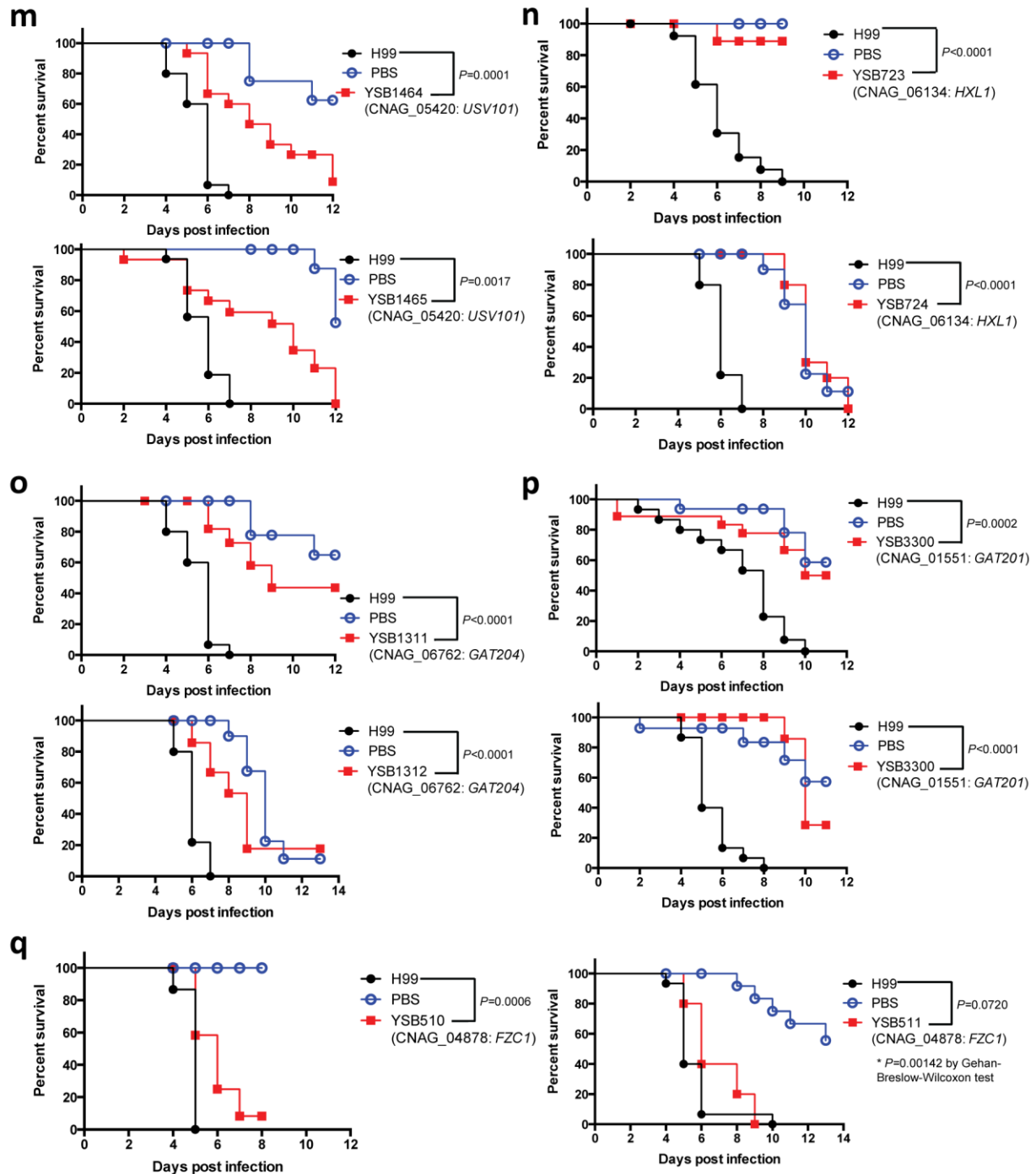


*Continued*

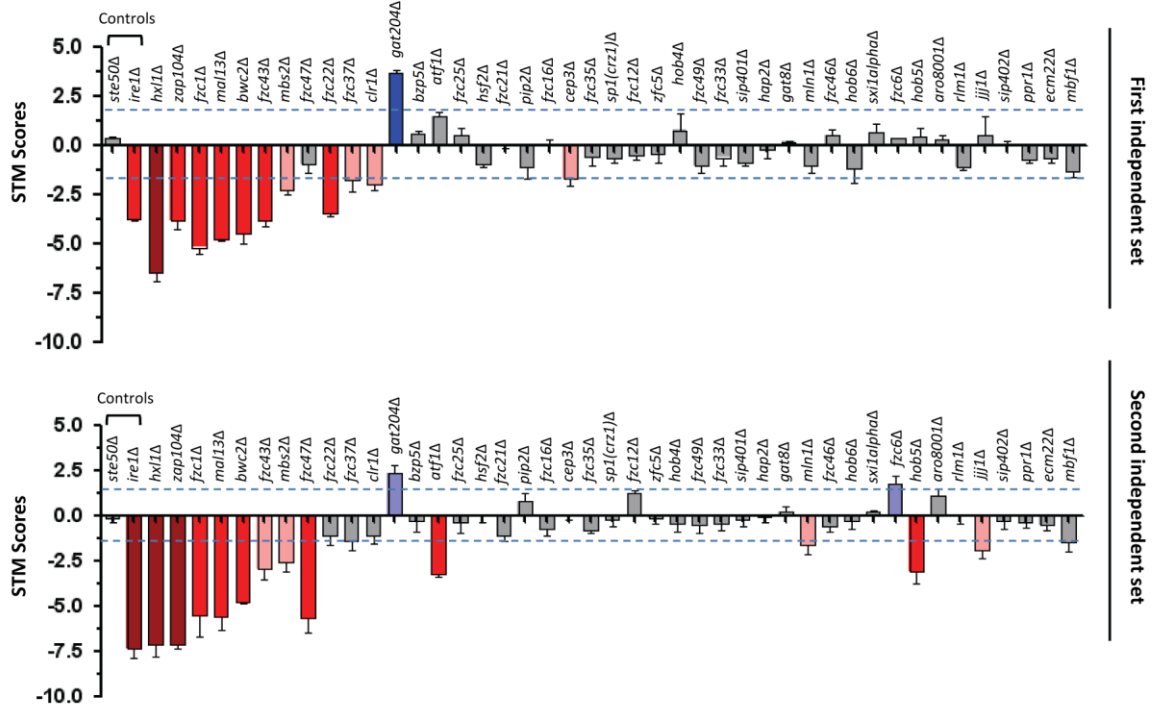
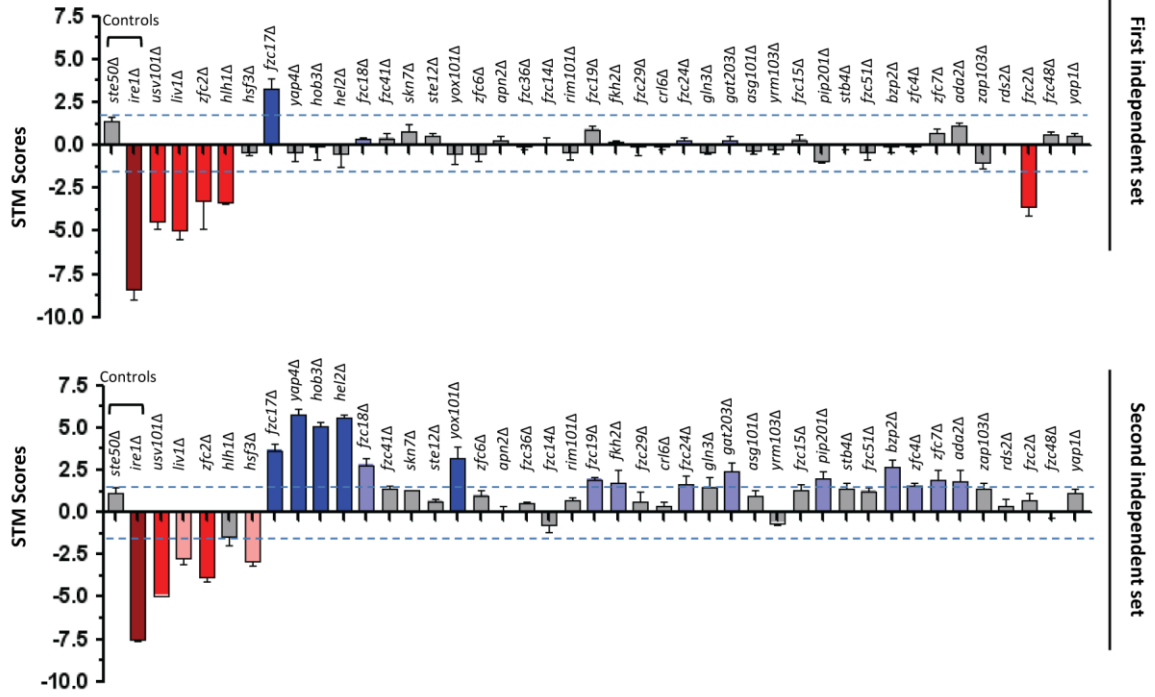


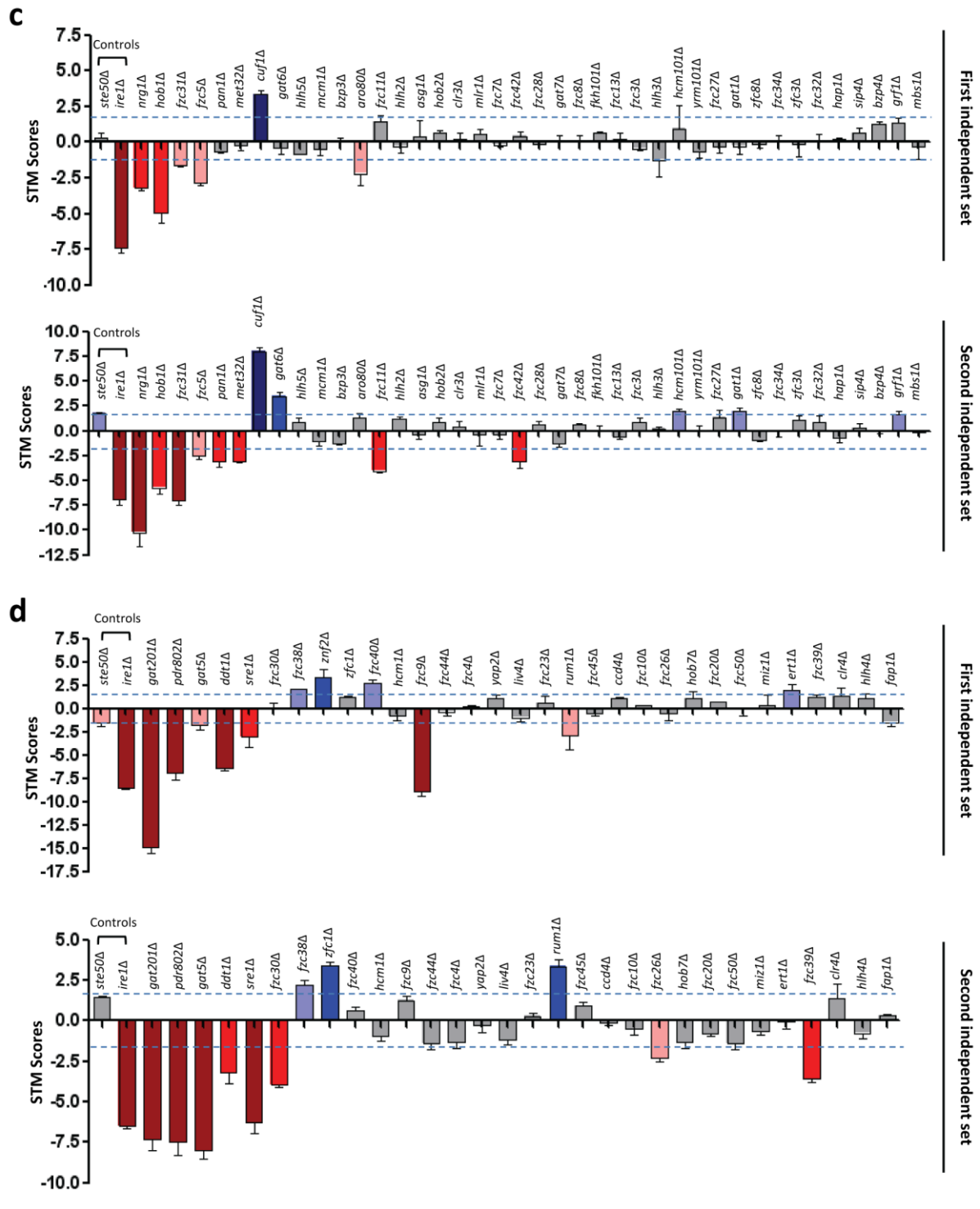
*Continued*





**Supplementary Figure 8: Identification of TFs involved in the virulence of *C. neoformans* by using the *Galleria mellonella* killing assay.** *C. neoformans* strains were grown overnight in liquid YPD medium, washed three times with phosphate-buffered saline (PBS), and inoculated into *G. mellonella* at the last larval stage at 4,000 cells/larva (15 larvae per group). The infected larvae were incubated at 37°C and their survival patterns were monitored up to 14 days. Statistical analysis was performed using the Log-rank (Mantel-Cox) test. Each panel (a to l) includes the survival data for two independent mutants for each TF.

**a****b***Continued*



**Supplementary Figure 9: Signature-tag mutagenesis (STM) scores of the *C. neoformans* TF mutants.** (a–d) STM scores of each TF mutant were measured by means of quantitative PCR analysis performed using a common primer and the signature tag-specific primers listed in Supplemental Table S1. Blue-dotted lines indicate STM score cutoffs ( $\pm 1.5$ ) above which we consider the change to be a meaningful STM change. The cutoff value was set based on the range of STM scores of the *ste50Δ* mutant, which is the virulent control strain. [Strain information: (a) set1: *hxl1Δ* (YSB723), *zap104Δ* (YSB2134), *fzc1Δ* (YSB510), *mal13Δ* (YSB506), *bwc2Δ* (YSB1839), *fzc43Δ* (YSB517), *mbs2Δ* (YSB538), *fzc47Δ* (YSB1406), *fzc22Δ* (YSB1688), *fzc37Δ* (YSB1329), *clr1Δ*



(YSB1396), *gat204Δ* (YSB1311), *bzp5Δ* (YSB1474), *atf1Δ* (YSB676), *fzc25Δ* (YSB518), *hsf2Δ* (YSB2295), *fzc21Δ* (YSB1252), *pip2Δ* (YSB1249), *fzc16Δ* (YSB2326), *cep3Δ* (YSB847), *fzc35Δ* (YSB1341), *sp1Δ/crz1Δ* (YSB1263), *fzc12Δ* (YSB467), *zfc5Δ* (YSB2177), *hob4Δ* (YSB1435), *fzc49Δ* (YSB2171), *fzc33Δ* (YSB1074), *sip401Δ* (YSB1358), *hap2Δ* (YSB1104), *gat8Δ* (YSB471), *mln1Δ* (YSB1172), *fzc46Δ* (YSB1209), *hob6Δ* (YSB1255), *sxi1alphaΔ* (YSB1390), *fzc6Δ* (YSB1980), *hob5Δ* (YSB1586), *aro8001Δ* (YSB661), *rlm1Δ* (YSB1300), *jjj1Δ* (YSB1592), *sip402Δ* (YSB529), *ppr1Δ* (YSB1046), *ecm22Δ* (YSB476), *mbf1Δ* (YSB768). set2: *hxl1Δ* (YSB724), *zap104Δ* (YSB2135), *fzc1Δ* (YSB511), *mal13Δ* (YSB507), *bwc2Δ* (YSB1840), *fzc43Δ* (YSB2334), *mbs2Δ* (YSB539), *fzc47Δ* (YSB1407), *fzc22Δ* (YSB1689), *fzc37Δ* (YSB1330), *clr1Δ* (YSB1397), *gat204Δ* (YSB1312), *bzp5Δ* (YSB1475), *atf1Δ* (YSB678), *fzc25Δ* (YSB1822), *hsf2Δ* (YSB2296), *fzc21Δ* (YSB1253), *pip2Δ* (YSB1250), *fzc16Δ* (YSB2327), *cep3Δ* (YSB848), *fzc35Δ* (YSB1342), *sp1Δ/crz1Δ* (YSB1264), *fzc12Δ* (YSB468), *zfc5Δ* (YSB2178), *hob4Δ* (YSB1437), *fzc49Δ* (YSB2173), *fzc33Δ* (YSB1075), *sip401Δ* (YSB1359), *hap2Δ* (YSB1105), *gat8Δ* (YSB472), *mln1Δ* (YSB1173), *fzc46Δ* (YSB1210), *hob6Δ* (YSB1256), *sxi1alphaΔ* (YSB1391), *fzc6Δ* (YSB1981), *hob5Δ* (YSB1585), *aro8001Δ* (YSB662), *rlm1Δ* (YSB1301), *jjj1Δ* (YSB1594), *sip402Δ* (YSB530), *ppr1Δ* (YSB1047), *ecm22Δ* (YSB478), *mbf1Δ* (YSB769). (b) set1: *usv101Δ* (YSB1464), *liv1Δ* (YSB2211), *zfc2Δ* (YSB2622), *hlh1Δ* (YSB1175), *hsf3Δ* (YSB2527), *fzc17Δ* (YSB2250), *yap4Δ* (YSB1587), *hob3Δ* (YSB2001), *hel2Δ* (YSB1382), *fzc18Δ* (YSB2320), *skn7Δ* (YSB349), *ste12Δ* (YSB1542), *yox101Δ* (YSB3134), *zfc6Δ* (YSB1953), *apn2Δ* (YSB1429), *fzc36Δ* (YSB2335), *fzc14Δ* (YSB1846), *rim101Δ* (YSB1366), *fzc19Δ* (YSB2115), *fkh2Δ* (YSB1339), *fzc29Δ* (YSB718), *crl6Δ* (YSB1106), *fzc24Δ* (YSB774), *gln3Δ* (YSB3154), *gat203Δ* (YSB569), *asg101Δ* (YSB2697), *ymr103Δ* (YSB2298), *fzc15Δ* (YSB646), *pip201Δ* (YSB3099), *stb4Δ* (YSB1013), *fzc51Δ* (YSB1842), *bzp2Δ* (YSB2702), *zfc4Δ* (YSB2231), *zfc7Δ* (YSB481), *ada2Δ* (YSB2381), *zap103Δ* (YSB2540), *rds2Δ* (YSB18989), *fzc2Δ* (YSB1050), *fzc48Δ* (YSB2646), and *yap1Δ* (YSB815). set2: *usv101Δ* (YSB1465), *liv1Δ* (YSB2212), *zfc2Δ* (YSB2623), *hlh1Δ* (YSB1176), *hsf3Δ* (YSB2528), *fzc17Δ* (YSB2251), *yap4Δ* (YSB1661), *hob3Δ* (YSB2002), *hel2Δ* (YSB1383), *fzc18Δ* (YSB2321), *skn7Δ* (YSB350), *ste12Δ* (YSB1543), *yox101Δ* (YSB3136), *zfc6Δ* (YSB1954), *apn2Δ* (YSB1430), *fzc36Δ* (YSB2523), *fzc14Δ* (YSB1847), *rim101Δ* (YSB1367), *fzc19Δ* (YSB2116), *fkh2Δ* (YSB1340), *fzc29Δ* (YSB719), *crl6Δ* (YSB1107), *fzc24Δ* (YSB775), *gln3Δ* (YSB3155), *gat203Δ* (YSB570), *asg101Δ* (YSB2698), *ymr103Δ* (YSB2299), *fzc15Δ* (YSB647), *pip201Δ* (YSB3100), *stb4Δ* (YSB1014), *fzc51Δ* (YSB1843), *bzp2Δ* (YSB2703), *zfc4Δ* (YSB2232), *zfc7Δ* (YSB482), *ada2Δ* (YSB2382), *zap103Δ* (YSB2541), *rds2Δ* (YSB1899), *fzc2Δ* (YSB1051), *fzc48Δ* (YSB2647), and *yap1Δ* (YSB1290). (c) set1: *nrg1Δ* (YSB3096), *hob1Δ* (YSB2308), *fzc31Δ* (YSB3093), *pan1Δ* (YSB1181), *met32Δ* (YSB1179), *cuf1Δ* (YSB2665), *gat6Δ* (YSB1384), *hlh5Δ* (YSB2609), *mcm1Δ* (YSB1302), *bzp3Δ* (YSB1099), *aro80Δ* (YSB714), *fzc11Δ* (YSB845), *hlh2Δ* (YSB1147), *asg1Δ* (YSB3013), *hob2Δ* (YSB2282), *clr3Δ* (YSB1834), *mlr1Δ* (YSB2727), *fzc7Δ* (YSB2704), *fzc42Δ* (YSB690), *fzc28Δ* (YSB2337), *gat7Δ* (YSB2699), *fzc8Δ* (YSB2112), *fkh101Δ* (YSB1856), *fzc13Δ* (YSB2517), *fzc3Δ* (YSB2611), *hlh3Δ* (YSB2329), *hcm101Δ* (YSB2390), *ymr101Δ* (YSB2997), *fzc27Δ* (YSB582), *gat1Δ* (YSB2973), *zfc8Δ* (YSB3031), *fzc34Δ* (YSB501), *zfc3Δ* (YSB2108), *fzc32Δ* (YSB2385), *hap1Δ* (YSB2481), *sip4Δ* (YSB2680), *bzp4Δ* (YSB1894), *grf1Δ* (YSB796), *mbs1Δ* (YSB488). set2: *nrg1Δ* (YSB3097), *hob1Δ* (YSB2309), *fzc31Δ* (YSB3094), *pan1Δ* (YSB1183), *met32Δ* (YSB1178), *cuf1Δ* (YSB2666), *gat6Δ* (YSB1386), *hlh5Δ* (YSB3059), *mcm1Δ* (YSB1303), *bzp3Δ* (YSB1100), *aro80Δ* (YSB715), *fzc11Δ* (YSB846), *hlh2Δ* (YSB1149), *asg1Δ* (YSB3014), *hob2Δ* (YSB2283), *clr3Δ* (YSB1836), *mlr1Δ* (YSB2728), *fzc7Δ* (YSB2705), *fzc42Δ* (YSB687), *fzc28Δ* (YSB2338), *gat7Δ* (YSB2700), *fzc8Δ* (YSB2113), *fkh101Δ* (YSB1855), *fzc13Δ* (YSB2518), *fzc3Δ* (YSB2664), *hlh3Δ* (YSB2330), *hcm101Δ* (YSB2391), *ymr101Δ* (YSB2998), *fzc27Δ* (YSB583), *gat1Δ* (YSB2972), *zfc8Δ* (YSB3032), *fzc34Δ* (YSB2979), *zfc3Δ* (YSB2386), *fzc32Δ* (YSB2526), *hap1Δ* (YSB2482), *sip4Δ* (YSB2681), *bzp4Δ* (YSB1895), *grf1Δ* (YSB797), *mbs1Δ* (YSB489). (d) set1: *gat201Δ* (YSB3300), *pdr802Δ* (YSB2387), *gat5Δ* (YSB3033), *ddt1Δ* (YSB1583), *sre1Δ* (YSB2493), *fzc30Δ* (YSB2447), *fzc38Δ* (YSB777), *znf2Δ* (YSB2740), *zfc1Δ* (YSB2573), *fzc40Δ* (YSB3088), *hcm1Δ* (YSB1850), *fzc9Δ* (YSB2984), *fzc44Δ* (YSB2182), *fzc4Δ* (YSB2724), *yap2Δ* (YSB1416), *liv4Δ* (YSB2089),

*fzc23Δ* (YSB3105), *rum1Δ* (YSB3164), *fzc45Δ* (YSB2221), *ccd4Δ* (YSB706), *fzc10Δ* (YSB3083), *fzc26Δ* (YSB3084), *hob7Δ* (YSB3026), *fzc20Δ* (YSB3128), *fzc50Δ* (YSB3131), *miz1Δ* (YSB2133), *ert1Δ* (YSB693), *fzc39Δ* (YSB1820), *clr4Δ* (YSB3282), *hlh4Δ* (YSB2244), and *fap1Δ* (YSB813). set2: *gat201Δ* (YSB3301), *pdr802Δ* (YSB2388), *gat5Δ* (YSB3034), *ddt1Δ* (YSB2633), *sre1Δ* (YSB2494), *fzc30Δ* (YSB2448), *fzc38Δ* (YSB777), *zfc1Δ* (YSB2574), *fzc40Δ* (YSB3088), *hcm1Δ* (YSB1851), *fzc9Δ* (YSB3266), *fzc44Δ* (YSB2181), *fzc4Δ* (YSB2725), *yap2Δ* (YSB1417), *liv4Δ* (YSB2089), *fzc23Δ* (YSB3106), *rum1Δ* (YSB3164), *fzc45Δ* (YSB2222), *ccd4Δ* (YSB707), *fzc10Δ* (YSB3368), *fzc26Δ* (YSB3085), *hob7Δ* (YSB3027), *fzc20Δ* (YSB3129), *fzc50Δ* (YSB3132), *miz1Δ* (YSB3366), *ert1Δ* (YSB694), *fzc39Δ* (YSB2621), *clr4Δ* (YSB3283), *hlh4Δ* (YSB2245), and *fap1Δ* (YSB817)].

Supplementary Table 1. Transcription factors involved in antifungal agent resistance in <i>C. neoformans</i>		
Antifungal agents	TF mutants showing increased resistance	TF mutants showing increased susceptibility
Azole (Fluconazole)	<b>HOB1</b> , HAP2, SKN7, <b>NRG1</b> , <b>MBS1</b> , PPR1, <b>JJJ1</b> , <b>HCM1</b> , ADA2, FZC9, GAT7, <b>ERT1</b> , FKH2, <b>ECM22</b> , DDT1, GAT5, YRM103, <b>CUF1</b> , FZC51, LIV4,	BZP3, HLH3, BZP1/HXL1, <b>SRE1</b> , RIM101, YAP2, HLH1, YAP4, PIP2, MIZ1, MLN1, HOB6, MBF1, MET32, FZC46, <b>YAP1</b> , FZC14, FZC2, HSF2, ZFC6, FZC45, FZC30, ASG1, STE12, LIV1, FZC22, FZC31, PAN1, BZP2, SP1/CRZ1, BZP5, SXI1alpha, FZC34, , FZC17, HLH2 <b>HOB1</b> , <b>MBS1</b> , <b>JJJ1</b> , <b>ERT1</b> , <b>ECM22</b> , GAT201, ZAP104, SP1/CRZ1, FZC6, BZP5, HLH1, PIP2, <b>HCM1</b> , BZP2, USV101, HOB4, STE12, HOB5, GRF1, HEL2, FZC45, ASG1, FZC22, HOB6, PAN1, <b>CUF1</b> , FZC49, FZC1, BWC2, FAP1, FZC44, FZC8, FZC23, GAT204, <b>NRG1</b> , PIP201, RIM101, HLH3, BZP3, MLN1, MET32, ZFC2, FZC31, RUM1, PDR802, FZC10, HLH2
Polyene (Amphotericin B)	<b>SRE1</b> , <b>YAP1</b> , FZC51, SKN7, CLR1, BZP4, ATF1, FZC4	NRG1, ZFC2, YAP1, MBS1, FZC6, YAP2, BZP3, <b>JJJ1</b> , HLH1, PIP2, APN2, FZC46, HAP2, FZC51, BZP5, HCM1, FZC19, BZP2, FZC44
5-flucytosine	HLH3, RIM101, GAT204, HOB3, FZC50, ZNF2, RDS2, FZC31	USV101, ADA2, YAP1, FZC6, HLH1, PIP2, FZC46, HAP2, BZP1/HXL1, FKH2, LIV1, YAP2, BZP2, FZC21, HLH3, YRM101, BZP5, GLN3, ZFC8, DDT1, FZC22, HOB6, RLM1, MLN1, PAN1, FZC35, YRM103, ZFC3, ASG1, FZC41, FZC43, FZC51, HAP1, MET32, FZC32
Phenylpyrrole Fungicide (Fludioxonil)	NRG1, <b>JJJ1</b> , SP1/CRZ1, SKN7, GAT7, FAP1, ZFC2, GAT204, ZNF2, HEL2, FZC50, SRE1	



**Supplementary Table 2.** Transcription factors involved in the virulence of *C. neoformans*

CNAG	Gene	Insect host	Murine host	Phenotypes			
		RMS [mt/wt]	STM score	Capsule	Melanin	Urease	Stress responses
<b>01551</b>	<b>GAT201</b>	<b>1.9*</b>	<b>-11.125↓↓↓</b>	↓↓↓	-	↑	Ox/ER/Cm/Hm
<b>03894</b>	<b>PDR802</b>	<b>1.73*</b>	<b>-7.212↓↓↓</b>	-	-	-	-
<b>06134</b>	<b>BZP1/HXL1</b>	<b>&gt;1.67*</b>	<b>-6.82↓↓↓</b>	-	-	-	Th/Gx/ER/Cw/Hm
05392	ZAP104	1.00	-5.528↓↓	↓↓↓	-	↓↓	-
<b>04878</b>	<b>FZC1</b>	<b>1.2*</b>	<b>-5.365↓↓</b>	↑↑	↑↑↑	↑	Th/Ox/Gx/Cm
02774	MAL13	0.92	-5.219↓↓	-	-	-	-
04583	DDT1	1.00	-4.832↓↓↓	-	-	-	Ox/ER
<b>01431</b>	<b>HOB1</b>	<b>1.46*</b>	<b>-4.95↓↓</b>	-	↓↓↓	-	Th/Os/Ox/Gx/ER/Cm/Cw
<b>02435</b>	<b>BWC2</b>	<b>1.5*</b>	<b>-4.663↓↓</b>	-	-	-	Ox/ER/Cm
<b>05420</b>	<b>USV101</b>	<b>1.5*</b>	<b>-4.684↓↓</b>	↑↑	↓↓↓	↑	Th/Ox/ER/Cm
<b>04804</b>	<b>SRE1</b>	<b>2.04*</b>	<b>-4.643↓↓</b>	↑↑	-	↓	Th/Ox/Gx/ER/Cm/Hm
00460	LIV1	1.00	-3.875↓↓	↓	-	-	Ox
<b>01973</b>	<b>ZFC2</b>	<b>1.34*</b>	<b>-3.554↓↓</b>	-	-	-	Ox/ER
04841	FZC43	1.00	-3.403↓↓	-	-	-	Os
04184	FZC47	0.83	-3.37↓↓	↓↓	-	-	Hm
<b>05222</b>	<b>NRG1</b>	<b>1.33*</b>	<b>-3.226↓↓</b>	↑↑	↑↑	-	Th/Os/Ox/Gx/ER/Cm/Cw
03132	FZC5	1.00	-2.832↓	-	-	-	-
00791	HLH1	1.00	-2.408↓↓	-	↑↑	↓↓	Ox/ER
01438	MBS2	1.00	-2.443↓	-	↓	-	Th/Ox/Hm
07011	FZC22	0.83	-2.31↓	-	-	-	Ox/Cm
04353	CLR1	0.83	-2.025↓	↑	-	-	Ox/ER/Cm/Hm
04457	FZC30	1.00	-1.95↓	↑↑	-	-	Th/Ox/Cm
01173	PAN1	1.00	-1.88↓	-	-	-	Os/Ox/ER/Cm
05642	FZC37	1.00	-1.813↓	-	-	-	Ox/Hm
05153	GAT5	1.00	-1.803↓	-	-	-	Th/Os/Ox/Gx/ER/Cm/Hm
00068	MET32	1.00	-1.67↓	-	-	-	Os/Ox/ER
04036	HSF3	1.12	-1.65↓	-	-	-	Hm/Cm
<b>03741</b>	<b>FZC31</b>	<b>1.17*</b>	<b>-1.608↓</b>	-	↑↑	-	Th/Os/Ox/ER/Cm
03336	FZC50	1.47*	-0.736	-	-	-	Ox/Cm/Hm
00156	SP1/CRZ1	1.37*	-0.485	-	-	-	Th/Ox/ER/Cm/Cw
00239	YAP1	1.15*	0.812	↓↓↓	↑↑	↑	Ox/ER/Cm/Hm
04263	BZP2	1.58*	1.29	-	-	-	Th/Os/Ox/Gx/ER/Cm/Cw/Hm
00514	GAT6	0.99	1.52↓	-	-	-	Th/Ox/Gx/ER/Cm
04012	FZC18	1.00	1.59↑	↑	-	-	-
01626	ADA2	>1.43*	1.448	↓↓↓	↑↑	-	Th/Os/Ox/Gx/ER/Hm
00841	FZC40	1.00	1.688↑	-	-	-	-
00830	FZC38	1.00	2.172↑	-	-	-	Ox
02603	ZFC1	1.70	2.29↑	-	-	-	Cm
03527	HEL2	0.83	2.49↑	-	-	-	Os/Ox/ER
05176	HOB3	1.00	2.53↑	↓	-	-	ER/Cm
07593	YAP4	0.90	2.64↑	-	-	-	ER
06762	GAT204	1.50*	2.986↑↑	↓↓	-	-	Ox/Hm
03366	ZNF2	1.00	3.351↑↑	-	-	-	Ox/Hm
02322	FZC17	0.85	3.443↑↑	↑	-	-	-
07724	CUF1	1.20	5.656↑↑	-	↓↓↓	-	Th/Os/Ox/ER/Cm/Cw/Hm

RMS [mt/wt], Relative median survival days = [median survival day for a mutant]/[median survival day for the wt strain (H99)]; \*,  $P < 0.05$ , by Log-rank (Mantel-Cox) test for mutant vs. wt strain. The RMS scores for two-independent strains for each TF mutant were described as an average value.

STM score, Signature tagged mutagenesis based quantitative PCR score =  $\text{Log}_2(\text{output/input})$  in the lung from the sacrificed mice (average score from three mice). (↓) or (↑) indicates reduced or enhanced virulence, respectively. Average STM scores from two independent mutants for each TF were described.

Capsule/Melanin/Urease, (-) indicates wt-like phenotypes, (↓) or (↑) indicates reduced or enhanced production, respectively. Single arrow (weak changes), Double arrows (moderate changes), Triple arrows (strong changes).

Os, Osmotic stress; Ox, Oxidative stress; Gx, Genotoxic stress; ER, ER-stress; Th, Thermal stress, Cm, Cell membrane stress, Cw, Cell wall stress, Hm, Heavy metal stress. Red and blue color letters indicate enhanced and reduced sensitivity, respectively, to each stress, while black letters indicate that mutants exhibit enhanced or reduced sensitivity to a corresponding stress depending on stress inducing agents.

Bold typed indicate TFs, whose deletions affected virulence at statistically significant levels in both insect and murine models of cryptococcosis.